Claims:

1. A method for development of nucleotide probes for myctophid fishes, said method comprising the steps of :

(i) extracting the DNA from the muscle tissue of a myctophid fish,

(ii) selecting gene regions in the extracted DNA with the selected primers and the amplifying the same using polymerase chain reaction (PCR),

(iii) eluting the PCR amplified DNA,

(iv) reamplifying the gene regions from PCR amplified DNA and eluting the same,

(v) cycle sequencing of eluted DNA using a single primer,

(vi) purifying extension products,

(vii) sequencing the extension product on acrylamide gel,

(viii) confirming the sequences for the target gene by Blast -Email,

(ix) ligating the eluted PCR products in a vector,

(x) preparing the electro-competent cells for electro transformation,

(xi) electro transforming the host cells,

(xii) growing and harvesting of transformed host cells,

(xiv) confirming that the transformed bacteria has the plasmids with the gene inserts by PCR.

(xv) purifying recombinant plasmid DNA having the cloned gene probes from the transformed host cells,

(xvi) checking purity and specificity of the cloned DNA probe insert by cutting with restriction enzyme,

(xvii) confirming the molecular size of the DNA probe insert,

(xviii) PCR amplification of the gene insert from the probe using both primers,

(xix) eluting of the amplified gene region,

(xx) cycle sequencing of the gene region of the probe,

(xxi) sequencing of the cloned DNA insert on acrylamide gel,

(xxii) comparing the DNA sequence of the prepared DNA probes using "BLAST program "against the known sequences of similar genes in the genome data bases,

(xxiii) confirming the sequences of the cloned probe by aligning with sequences of the claim 1(vii), and

(xxiv) designing species specific primers from the sequences.

2. A method as claimed in claim 1 wherein the myctophid fishes are selected from the group comprising *Stenobrachis leucopsarus*, *Diaphus theta, Protomyctophum crockeri, Tarletonbeania crenularis and Lampanyctus regalis.*

3. A method as claimed in claim 1 wherein the gene regions are selected from mitochondrial and nuclear genes.

4. A method claimed in claim 1 wherein the mitochondrial genes taken for probe preparation are selected from the group comprising: Cyt b and D-loop genes, 12 S RNA and 16 S RNA genes.

5. A method claimed in claim 1 wherein the nuclear genes taken for probe preparation are selected from Rod and ITS-2 genes.

6. A method of claim 1 wherein the PCR amplified cleaned nuclear gene probe is Rod gene.

7. A method claimed in claim 1 wherein the nuclear gene taken for the cloned probe preparation is ITS-2 gene.

8. A method as claimed in claim 1 wherein the concentration of primers used for PCR amplification is 20 meu. L.

9. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for amplification and detection of Cyt b gene contains oligonucleotides with the sequences:

CYT 1: 5' TGA YTT GAA RAA CCA YCG TTG 3'

CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

10. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for reamplification and detection of Cyt b gene contains oligonucleotides with the sequences:

CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'

CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

11. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification and detection of D-Loop gene contains oligonucleotides with the sequences :

PRO-L : 5' CTA CC 3'

D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3'

12. A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of ITS2 gene were

ITS1 F : 5' TTG TAC ACA CCGCCCGTC GC 3'

ITS2 R : 5' ATA TGC TTA AAT TCA GCG GG 3'

13. A method as claimed in claim 1 wherein the forward and backward primers used for PCR reamplification of ITS2 gene from ITS1 F and ITS2 R PCR amplification were

ITS2 F: 5' CTA CGC CTG TCT GAG TGT C 3'

ITS2 R: 5' ATA TGC TTA AAT TCA GCG GG 3'

14. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of Rhodopsin gene Rod contains oligonucleotides with the sequences:

ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'

ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'

15. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene contains oligonucleotides with the sequences:

12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'

12 SB-H : 5 ' AGA GTG ACG GGC GGT GTG T 3'

16. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene contains oligonucleotides with the sequences:

16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'

16 SBR-H : 5 ' CCG GTC TGA ACT CAG ATC ACG T 3'

17. A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of Rhodopsin gene Rod were :

ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'

ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'

18. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene were :

12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'

12 SB-H: 5 ' AGA GTG ACG GGC GGT GTG T 3'

19. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene were :

16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'

SBR-H: 5 ' CCG GTC TGA ACT CAG ATC ACG T 3'

20. A method claimed in claim 1 wherein the 12S RNA gene and 16S RNA gene in the myctophid fish *Stenobrachius leucopsarus* were amplified by PCR.

21. A method claimed in claim 1 wherein the 12S RNA and 16S RNA gene in myctophid fish *Diaphus theta* were eluted by PCR amplification.

22. A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 12 S RNA.

23. A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 16 S RNA.

24. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 12 S RNA.

25. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 16 S RNA.

26. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 12 S RNA.

27. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 16 S RNA.

28. A method claimed in claim 1 wherein the cycle sequencing primer concentration used was 2μL,

29. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:

CYT 1: 5' TGA YTT GAA RAA CCA YCG TTG 3'

30. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:

CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

31. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:

CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'

32. A method claimed in claim 1 wherein the cycle sequencing forward primer for D-Loop region consisted of oligonucleotides with the sequence:

PRO-L : 5' CTA CC 3'

33. A method claimed in claim 1 wherein the backward cycle sequencing primer for D-Loop region consisted of oligonucleotides with the sequence:

D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3'

34. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:

ITS 1 -F : 5' TTG TAC ACA CCG CCC GTC GC 3'

35. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:

ITS2 –R : 5' ATA TGC TTA AAT TCA GCG GG 3'

36. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of Rhodopsin gene Rod consisted of oligonucleotides with the sequence:

ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'

37. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing consisted of oligonucleotides with the sequence:

ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'

38. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:

12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'

39. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:

12 SB-H : 5 ' AGA GTG ACG GGC GGT GTG T 3'

40. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:
16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'

41. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:
16 SBR-H: 5 ' CCG GTC TGA ACT CAG ATC ACG T 3'

42. A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region are purified by conventional methods.

43. A method as claimed in claim 1 wherein the extension products of 16 S gene region are purified by conventional method.

44. A method as claimed in claim 1 wherein the extension products of CYT b gene are purified by conventional method.

45. A method as claimed in claim 1 wherein the extension products of ROD gene are purified by conventional method.

46. A method as claimed in claim 1 wherein the extension products of D-Loop control region are purified by conventional method.

47. A method as claimed in claim 1 wherein the extension products of ITS2 region are purified by conventional method.

48. A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region was sequenced in an automated sequencer.

49. A method as claimed in claim 1 wherein the extension products of 16 S gene region was sequenced in an automated sequencer.

50. A method as claimed in claim 1 wherein the extension products of CYT b gene was sequenced in an automated sequencer.

51. A method as claimed in claim 1 wherein the extension products of ROD gene was sequenced in an automated sequencer.

52. A method as claimed in claim 1 wherein the extension products of D-Loop control region was sequenced in an automated sequencer.

53. A method as claimed in claim 1 wherein the extension products of ITS2 region was sequenced in an automated sequencer.

54. A method as claimed in claim 1 wherein the identity of the gene 12S RNA is confirmed by Blast Email.

55. A method as claimed in claim 1 wherein the identity of the gene 16S RNA is confirmed by Blast Email.

56. A method as claimed in claim 1 wherein the identity of the gene CYT b is confirmed by Blast Email.

57. A method as claimed in claim 1 wherein the identity of the gene ROD is confirmed by Blast Email.

58. A method as claimed in claim 1 wherein the identity of the D-Loop is confirmed by Blast Email.

59. A method as claimed in claim 1 wherein the identity of the gene ITS2 is confirmed by Blast Email.

60. A method as claimed in claim 1 wherein the vector used for cloning was Bluescript KS^- phagemid.

61. A method as claimed in claim 1 wherein the vector used for cloning had ampicillin resistance gene for selection.

62. A method as claimed in claim 1 wherein the vector used for cloning had Lac Z gene for blue white colony selection.

63. A method as claimed in claim 1 wherein the CoI E 1 was the origin for replication of phagemid in the absence of helper phage.

64. A method as claimed in claim 1 wherein F 1 (-) origin for recovery of antisense strand of lac Z gene when a host strain containing the bluescript II phagemid.

65. A method as claimed in claim 1 wherein the host cells used for transformation were E. coli blue bacteria (Bacteria Strain XL 1 blue) XL1-Blue :- F' ::Tn10,pro $A^+B^+lacI^q$ (lacZ)M15/recA1endA1gyrA96(Nal^r)thi hsdR17(r_k^- m_k^+)supE44relA1 lac.

66. A method as claimed in claim 1 wherein probes are containing oligonucleotide sequences are cloned Cyt b , D-Loop, ITS2 and Rod genes.

67. A method as claimed in claim 1 wherein the probes of CYT b gene is an oligonucleotide sequence named as PSL CYTL.

68. A method as claimed in claim 1 wherein the probes of ITS 2 gene is an oligonucleotide sequence named as PSL ITS 2F.

69. A method as claimed in claim 1 wherein the probes of D-Loop control region gene is an oligonucleotide sequence named as PSL PROL.

70. A method as claimed in claim 1 wherein the PCR amplified sequence of ROD gene probe is named as ROD SLMB.

71. A method as claimed in claim 1 wherein the PCR amplified sequence of D-Loop gene probe is named as D-Loop SLMB.

72. A method as claimed in claim 1 wherein the PCR amplified sequence of ITS 2 gene probe is named as ITS 2 SLMB.

73. A method as claimed in claim 1 wherein the PCR amplified sequence of Cyt b gene probe is named as Cyt L SLMB.

74. The nucleotide base sequences of PSL CYTL (748 bp) comprising :

5'

CTTNCCCATT TTGGGCGCTT NGGCNCGCTN CTCCNCGAGA CTCTGCGTAN
TAATCCAANT CNCTNCGGGC CNCTCCCTAC CANTNCNCTA CACCNCAAAT
TNCAACCCNG TTTCCTCATC ANTCAACCAC ATCTGTCGAA AACNTCAACT
ACGGCTGACT AATCCGAAAA CATGCACGCT AACGGTGCCT CTTTCTTCTT
CATCTGTATT TATCTNCNCN TTGGANGAGG ACTATNCTAC GGATCCTACC
TCTACGAAGA GACGTGAGGT GTTGGTGTTA TTCTTCTCCT TCTAATAATG
ATGACTGCNT TTGTTGGCTA TGTGCTNCCC NGAGGACAAA TGTCCTTTTG
AGGTGCTACT GTCATTACAA NCCTACTCTC TGCTGTNCCG TNTGTTNGCG
GCNCTCTANT TCAATGAATT TGAGGTGGCT TCTCCGTAAA CACGCAACGC
TCACTCGTTT CTTCGCNTTC CACTTCTTGT TCCCATTTGT TGTCGCNGCT
ATAACCNNGG TTCACCNGAT TTNCCGACAT CAAACAGGCT CTAAANCCCC
CCCGGNTTGA CTCCATACAA CAAAACCCTC CACCCTATTC NCTATAAAAC
TCTAGGTTCG TGCCCGTATT GGCTTACTTC ATGNCTATTT CCCNGNCGGA
GGGACNAAAA TTCCTGCACC CCCTCCCCNC AAAATAAANA ATGTGTCTNT
CCTACCANAA AACAACNNAN ACGGGGTNTG CNCTTCCATC ATCCACN 3'

75. The nucleotide base sequences of PSLITS2F comprises :(225BP)

5'

TCTACGATCT	ACCGGCNTTT	NNTGTGGAAA	GACGATCATG
CATTTATGTG	TGTCTTTCTA	TGGATTTGAA	CCGTGTGGTA
CGTCTTTGCG	TACTGCTTGG	AAGGCTCAAC	TTGCTTCTGT
CCTTCTCTTG	CAGTCTCGCA	CTGTCTATGC	AACGTGTTCT
ACTTCGACTT	CTGTCGAAAA	ATCTTACTTT	TGACCTCAGA
TCAGACAAGA	CTACCCGCTG	AATTT 3'	

76. The nucleotide base sequences of PSL PROL comprises :(749 BP)

5'

CCTTTTCGGN	ATAGGCCCAN	CTCAAATGAA	TTCCTTCTCT
CCTGGTCCAA	GCCCAAACTG	TGGACGGCAG	GTTGACAATG
GTTACAAATC	GTGACAAATC	GGCTACATAA	TTGCCGATAG
CGATGTCGTC	AAACCAAGTC	AAACAATGGC	CGATGTATAT
CGGCCAAACC	CATATATGGG	TCTGGCTGTA	GTTTGTGTTG
AGCAACGTCA	CACCAGTGTC	TGGTCAGCAT	ATAAGATGTT
GACATCTTGC	AACATCTTAC	CCACAGACAG	ACAGTTACGG
CTGCTTACGA	ANGGCGCTAG	TGTTGTGGTG	AGAAACGAAG
ATACATACGT	CAAACAGACG	CCGGTGCACT	TGAAGACACT
GTTTGAAGGT	GCCGCACTAC	TTGACAGACA	GCCCATGATG
CGCTGGACAG	TGACCAAAGC	TACNGGAGGA	CCANATGGAA
ATCCTGTTGG	CGTTGCCGTG	GGACTCAAGT	TGTACACTTT
TGGATGGTTG	ATCACTANAN	CCGCTGCCGG	GAGAAGCACT
CGCTCCTGGT	TCACTAATCA	GATTGAGGTT	AACCANATTG
ANGTAAACAT	CTTCAACACA	GTGTCTTTAT	GCTGGATGAA
ATTNAGCCCA	CNGGACACCA	NAAAAGAATT	NCCNCTGGTT
CTNNCGGGGG	NCCCCNNNAA	CGNNTNTTCC	CCTTNTCTCN
NNNGCGGNGA	AGTTNCCCCC	CCCCACTNAN	NTCTTCCTTC
AANANNTTTC	CNCCNNNAGA	GGTTTTCCCN	3'

77. The nucleotide base sequences of ROD PSL SLMB comprises: (748 BP)

5'

CCTGGTAGGG	TTCCCCGTCA	ACTTCCTCAC	ACTGTACCTC
ACNTTCGAGC	ACAAGAAGCT	ACTAACCCCC	TTAAACTACA
TCCTGCTCAA	CCTGGCGGTC	GGAGACCTCC	TGATGGTGTA
AGGAGGGTTC	ACCACCACCA	TCTACACCTC	CATGCACGGC
TACTTCGTCC	TAGGGAAACT	GGGCTGCGCC	ATCGAAGGTT

TCATGGCCAC	CCATGGTGGT	CAGGTCGCCC	TTTGGTCCCT
GGTGGTTTTG	GCCGTGGAAA	GGTGGCTGGT	CGTCTGCAAN
CCCATCTCCA	GCTTCCGCTT	CCAGGAGTCC	CACTCCCTCA
TGGGCCTGGC	CGTGACCTGG	GTGATGGCGA	CGGCTTGTTC
TGTGCCCCCC	CTGGGTCGGC	TGGTCTCGCT	ACATCCCAGA
AGGCATGCAG	TGCTCATGCG	GAATGGACTA	CTACACTCCC
GCGCCGGGCG	TCAACAATGA	ATCCTACGTN	GTGTACATGT
TCNTCANAAA	AANAATNGGA	CCNCNGGGCG	ATCATNTTGN
TANGNNAAGG	CCAGNTGNTG	NGAGCAGTCA	AGGCGGCCGC
CGCCGCCCAG	CAAGAGTCCG	AGACCACCCA	GAGGGCCGAG
AGGGAAGTCA	CCCGNATGGT	NATNANGATG	GTNATNTCNT
TCNTGGTAAG	NAGGGNGCCA	NACGCCAGCG	TGGCCTGGTG
GATCTTNNGN	AACCAGGGNG	CAGAATTAGG	CCCNGTNTTC
ATGACCCTGC	CGGCNTTCTT	TGCCAAGA	3'

78. A method as claimed in claim 1 wherein FORWARD (L) primers of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:

5' CAA CCT CAT CTG TCG TAA AC 3'

and having the following characteristics:

i. is a 20-mer DNA oligonucleotide (sense),

ii. has melting temperature of 56.4 degree celius,

iii. has a molecular weight of 6101.0,

iv. has no hairpin loops,

v. has no single dimers,

vi. has no other dimers,

vii. has no single bulge loops or internal loops, and

viii. has no palindromes.

79. A method as claimed in claim 1 wherein BACKWARD (H) primer of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonuleotide comprising :

5' GCT CGG GCT GCT GGA ATC TT 3'

and having the following characteristics:

i. is a 20-mer DNA

ii. is an antisense oligonuleotide

iii. has a melting point of 70.8 degree celcius.

iv. has a molecular weight of 6220.1.

v. has no hairpin loops, no single bulge loops, no other internal loops, no single internal loops, no other bulge loops or palindromes.

vi. no single dimers or other dimers.

80. A method as claimed in claim 1 wherein forward primer of ITS2 F gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonuleotide comprising:

5' ACT TGA CTG ACC TTC TTA CT 3'

and having the following characteristics:

i. is a 20-mer sense oligonucleotide,

ii. has a melting point of 51.3 degree celcius,

iii. has a molecular weight of 6098.0,

iv. has no palindromes, loops and dimers,

81. A method as claimed in claim 1 wherein forward primer of ITS2 H gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonuleotide comprising :

5' ATA CTC TGC GGA CAT ACT TGA CTG 3'

and having the following characteristics:

i. is a 24-mer antisense oligonucleotide,

ii. has a melting point of 65.4 degree celcius.

iii. has a molecular weight of 7407.9.

iv. has no palindromes, loops and dimers.

82. A method as claimed in claim 1 wherein forward primer of pro-L for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonuleotide comprising:

5' CAG TCT CGT CAA ACC AAG TCA AAC 3'

and having the following characteristics:

i. is a 24-mer sense oligonucleotide

ii. has a melting point of 67.8 degree celcius.

iii. has a molecular weight of 7354.9.

iv. has no palindromes, loops and dimers.

83. A method as claimed in claim 1 wherein backward primer for Dloop for mitochondrial control region (dloop H) gene region for myctophid fish *Stenobrachius leucopsarus* is an oligonuleotide comprising :

5' ATA ATC ATC CAG CAT AAA CAC AC 3'

and having the following characteristics:

i. is a 23-mer antisense oligonucleotide,

ii. has a melting point of 61.2 degree celcius.

iii. has a molecular weight of 7033.7.

iv. has no palindromes, loops and dimers.

84. A method as claimed in claim 1 wherein the FORWARD primer (ROD- L) for Rhodopsin gene region of myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:

5' CCT GGT AGA GTT CGC CGT CA 3'

and having the following characteristics:

i. is a 20-mer sense oligonucleotide

ii. has a melting point of 67.4 degree celcius.

iii. has a molecular weight of 6189.0.

iv. has no palindromes, loops and dimers.

85. A method as claimed in claim 1 wherein the backward primer (ROD- H) for Rhodopsin gene region of myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:

5' CGT GTT CCT TAT CAT TGT GCC T 3'

and having the following characteristics:

i. is a 22-mer antisense oligonucleotide

ii. has a melting point of 66.4 degree celcius.

iii. has a molecular weight of 6738.4.

iv. has no palindromes, loops and dimers.

86. A method as claimed in claim 1 wherein the forward primer of 16S-L of the myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:

5' CAC CAG CCA AGT ATG TTT CTC 3'

and having the following characteristics:

i. is a 21-mer sense oligonucleotide

ii. has a melting point of 61.5 degree celcius.

iii. has a molecular weight of 6421.4.

iv. has no palindromes, loops and dimers.

87. A method as claimed in claim 1 wherein the backward primer of 16s rRNA of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:

5' TCG TAG TTC AGC AGT CAG 3'

and having the following characteristics:

i. is a 18-mer antisense oligonucleotide

ii. has a melting point of 51.2 degree celcius.

iii. has a molecular weight of 5594.7.

iv. has no palindromes, hairpin loops and dimers.

88. A method as claimed in claim 1 wherein the forward primer 16S-L of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:

5' CTA TTC GCC TCG CTC AGA C 3'

and having the following characteristics:

i. is a 19-mer sense oligonucleotide

ii. has a melting point of 62.1 degree celcius.

iii. has a molecular weight of 5779.8.

iv. has no palindromes, hairpin loops and dimers.

89. A method as claimed in claim 1 wherein a primer 12S-H for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising:

5' GCC TCC ATC ATC CCT CAC CTT AC 3'

and having the following characteristics:

i. is a 23-mer antisense oligonucleotide

ii. has a melting point of 70.8 degree celcius.

iii. has a molecular weight of 6895.5

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

90. A method as claimed in claim 1 wherein the primer 12S-L for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising:

5' CTA TTC GCC TCG CTC AGA C 3'

and having the following characteristics:

i. is a 19-mer sense oligonucleotide

ii. has a melting point of 62.1 degree celcius.

iii. has a molecular weight of 5779.8

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

91. A method as claimed in claim 1 wherein 16S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:

5' AAA TCC GCC CTT ATG TGT GTT C 3'

and having the following characteristics:

i. is a 22-mer sense oligonucleotide

ii. has a melting point of 67.9 degree celcius.

iii. has a molecular weight of 6756.4

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

92. A method as claimed in claim 1 wherein 16S-H backward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' CTC CGT CCG TCT CGC CTC TG 3'
and having the following characteristics:
i. is a 20-mer antisense oligonucleotide
ii. has a melting point of 71.7 degree celcius.
iii. has a molecular weight of 6052.0
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

93. A method as claimed in claim 1 wherein 12S-H forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' CAT CGG CTT GCT CTA TTC CTT G 3'
and having the following characteristics:
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 68.8 degree celcius.
iii. has a molecular weight of 6723.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

94. A method as claimed in claim 1 wherein 12S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' TCT ATC GGC GGC GTA TCA C 3'
and having the following characteristics:
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 65.8 degree celcius.
iii. has a molecular weight of 5859.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

95. A method as claimed in claim 1 wherein 16S-H primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:

5' GGC GAT TCT ACG GCA CGG GCG 3'

and having the following characteristics:

i. is a 21-mer antisense oligonucleotide

ii. has a melting point of 80.4 degree celcius.

iii. has a molecular weight of 6568.3

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

96. A method as claimed in claim 1 wherein 16S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:

5' AAA CTG GTC CTC AAC TAT GTC A 3'

and having the following characteristics:

i. is a 22-mer sense oligonucleotide

ii. has a melting point of 60.7 degree celcius.

iii. has a molecular weight of 6758.5

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

97. A method as claimed in claim 1 wherein 16S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:

5' GGC GAT TCT ACG GCA CGG GCG 3'

and having the following characteristics:

i. is a 21-mer antisense oligonucleotide

ii. has a melting point of 80.4 degree celcius.

iii. has a molecular weight of 6568.3

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

98. A method as claimed in claim 1 wherein 12S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:

5' CCG ATT CAG CCA CGA TTC CCT C 3'

and having the following characteristics:

i. is a 22-mer antisense oligonucleotide

ii. has a melting point of 74.6 degree celcius.

iii. has a molecular weight of 6671.4

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

99. A method as claimed in claim 1 wherein 12S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:

5' CCT AAA GCC CAG ATA ACT ACA 3'

i. is a 21-mer sense oligonucleotide

ii. has a melting point of 59.2 degree celcius.

iii. has a molecular weight of 6432.3

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

100. A method as claimed in claim 1 wherein 16S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:

5' CGT GTT CTG ATG ATG ATG TGC T 3'

i. is a 22-mer antisense oligonucleotide

ii. has a melting point of 64.7 degree celcius.

iii. has a molecular weight of 6867.5

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

101. A method as claimed in claim 1 wherein 16S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:

5' ATT CCT TCC TCT TAG TAT G 3'

i. is a 19-mer sense oligonucleotide

ii. has a melting point of 49.5 degree celcius.

iii. has a molecular weight of 5799.8

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

102. A method as claimed in claim 1 wherein 12S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:

5' GCT GAA CTT ACT ATG CCC TAC T 3'

i. is a 22-mer antisense oligonucleotide

ii. has a melting point of 60.3 degree celcius.

iii. has a molecular weight of 6725.4

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

103. A method as claimed in claim 1 wherein 12S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:

5' CCG ATT GAC GCC GAA CTA TG 3'

i. is a 20-mer sense oligonucleotide

ii. has a melting point of 68.1 degree celcius.

iii. has a molecular weight of 6182.1

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

104. A method as claimed in claim 1 wherein 16S-H backward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' TAC GCA TAA CGG CTC TGG 3'

i. is a 18-mer DNA oligonucleotide (Antisense)

ii. has a melting point of 61.4 degree celcius.

iii. has a molecular weight of 5579.7

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

105. A method as claimed in claim 1 wherein 16S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' CTA CTA CAC CTC AAC TAC ATC T 3'

i. is a 22-mer sense oligonucleotide

ii. has a melting point of 52.4 degree celcius.

iii. has a molecular weight of 6638.4

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

106. A method as claimed in claim 1 wherein 12S-H forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' CCC ACT CAC TGC TAA CTC C 3'

i. is a 19-mer sense oligonucleotide

ii. has a melting point of 58.4 degree celcius.

iii. has a molecular weight of 5708.8

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

107. A method as claimed in claim 1 wherein 12S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' GGC TAA CTA CAA TCA TCT GCT 3'

i. is a 21-mer sense oligonucleotide

ii. has a melting point of 58.5 degree celcius.

iii. has a molecular weight of 6445.2

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Analysis of "table1 (slmb primer cyt L)" a 20-mer DNA Oligonucleotide(Sense)

5' CAA CCT CAT CTG TCG TAA AC 3'

Oligonucleotide Analysis

Molecular weight	6101.0	
Tm thermodynamic	56.4	degrees C
Filter Tm	48.8	degrees C
% GC Tm	66.2	degrees C
AT+GC Tm	58.0	degrees C
Absorbance	5.3	nMol/A260
Absorbance	32.5	ug/A260
Percent GC	45.0	%
Delta G	-28.7	kCal/Mol
Delta H	-140.6	kCal/Mol
Delta S	-368.0	eu
3' End Delta G	-5.9	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 2 (slmb primer cyt H)" a 20-mer DNA Oligonucleotide(Antisense)

5' GCT CGG GCT GCT GGA ATC TT 3'

Oligonucleotide Analysis

Molecular weight	6220.1	
Tm thermodynamic	70.8	degrees C
Filter Tm	63.2	degrees C
% GC Tm	72.3	degrees C
AT+GC Tm	64.0	degrees C
Absorbance	5.6	nMol/A260
Absorbance	34.8	ug/A260
Percent GC	60.0	%
Delta G	-37.5	kCal/Mol
Delta H	-164.6	kCal/Mol
Delta S	-419.9	eu
3' End Delta G	-5.1	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 3 (slmb primer ITS2 F)" a 20-mer DNA Oligonucleotide (Sense)

5' **ACT TGA CTG ACC TTC TTA CT** 3'

Oligonucleotide Analysis

Molecular weight	6098.0	
Tm thermodynamic	51.3	**degrees C**
Filter Tm	43.7	**degrees C**
% GC Tm	64.2	**degrees C**
AT+GC Tm	56.0	**degrees C**
Absorbance	5.6	**nMol/A260**
Absorbance	34.0	**ug/A260**
Percent GC	40.0	**%**
Delta G	-26.5	**kCal/Mol**
Delta H	-137.7	**kCal/Mol**
Delta S	-365.8	**eu**
3' End Delta G	-3.9	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 4 (slmb primer ITS2-H)" a 24-mer DNA Oligonucleotide(Antisense)

5' **ATA CTC TGC GGA CAT ACT TGA CTG** 3'

Oligonucleotide Analysis

Molecular weight	7407.9	
Tm thermodynamic	65.4	degrees C
Filter Tm	57.8	degrees C
% GC Tm	72.2	degrees C
AT+GC Tm	70.0	degrees C
Absorbance	4.4	nMol/A260
Absorbance	32.4	ug/A260
Percent GC	45.8	%
Delta G	-35.5	kCal/Mol
Delta H	-169.5	kCal/Mol
Delta S	-442.0	eu
3' End Delta G	-5.2	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 5 (slmb primer pro-L) " a 24-mer DNA Oligonucleotide(Sense)

5' CAG TCT CGT CAA ACC AAG TCA AAC 3'

Oligonucleotide Analysis

Molecular weight	7354.9	
Tm thermodynamic	67.8	degrees C
Filter Tm	60.2	degrees C
% GC Tm	72.2	degrees C
AT+GC Tm	70.0	degrees C
Absorbance	4.3	nMol/A260
Absorbance	31.4	ug/A260
Percent GC	45.8	%
Delta G	-36.5	kCal/Mol
Delta H	-169.9	kCal/Mol
Delta S	-439.7	eu
3' End Delta G	-4.9	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 6 (slmb primer Dloop-H)" a 23-mer DNA Oligonucleotide(Antisense)

5' **ATA ATC ATC CAG CAT AAA CAC AC** 3'

Oligonucleotide Analysis

Molecular weight	7033.7
Tm thermodynamic	61.2 degrees C
Filter Tm	53.6 degrees C
% GC Tm	66.4 degrees C
AT+GC Tm	62.0 degrees C
Absorbance	4.3 nMol/A260
Absorbance	30.0 ug/A260
Percent GC	34.8 %
Delta G	-32.9 kCal/Mol
Delta H	-163.3 kCal/Mol
Delta S	-429.7 eu
3' End Delta G	-4.6 kCal/Mol

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 7 (slmb primer ROD-L)" a 20-mer DNA Oligonucleotide (Sense)

5' CCT GGT AGA GTT CGC CGT CA 3'

Oligonucleotide Analysis

Molecular weight	**6189.0**	
Tm thermodynamic	**67.4**	**degrees C**
Filter Tm	**59.8**	**degrees C**
% GC Tm	**72.3**	**degrees C**
AT+GC Tm	**64.0**	**degrees C**
Absorbance	**5.3**	**nMol/A260**
Absorbance	**33.0**	**ug/A260**
Percent GC	**60.0**	**%**
Delta G	**-34.7**	**kCal/Mol**
Delta H	**-154.3**	**kCal/Mol**
Delta S	**-394.4**	**eu**
3' End Delta G	**-9.6**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 8 (slmb primer ROD-H)" a 22-mer DNA Oligonucleotide (Antisense)

5' **CGT GTT CCT TAT CAT TGT GCC T** 3'

Oligonucleotide Analysis

Molecular weight	**6738.4**	
Tm thermodynamic	**66.4**	**degrees C**
Filter Tm	**58.8**	**degrees C**
% GC Tm	**69.5**	**degrees C**
AT+GC Tm	**64.0**	**degrees C**
Absorbance	**5.2**	**nMol/A260**
Absorbance	**34.9**	**ug/A260**
Percent GC	**45.5**	**%**
Delta G	**-35.4**	**kCal/Mol**
Delta H	**-165.0**	**kCal/Mol**
Delta S	**-427.3**	**eu**
3' End Delta G	**-7.9**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 9 (LRMB primer 16S-L)" a 21-mer DNA Oligonucleotide(Sense)

5' **CAC CAG CCA AGT ATG TTT CTC** 3'

Oligonucleotide Analysis

Molecular weight	**6421.2**	
Tm thermodynamic	**61.5**	**degrees C**
Filter Tm	**53.9**	**degrees C**
% GC Tm	**68.9**	**degrees C**
AT+GC Tm	**62.0**	**degrees C**
Absorbance	**5.1**	**nMol/A260**
Absorbance	**33.0**	**ug/A260**
Percent GC	**47.6**	**%**
Delta G	**-31.9**	**kCal/Mol**
Delta H	**-152.3**	**kCal/Mol**
Delta S	**-396.4**	**eu**
3' End Delta G	**-4.9**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 10 (LRMB primer 16S-H)" a 18-mer DNA Oligonucleotide(Antisense)

5' TCG TAG TTC AGC AGT CAG 3'

Oligonucleotide Analysis

Molecular weight	**5594.7**	
Tm thermodynamic	**51.2**	**degrees C**
Filter Tm	**43.6**	**degrees C**
% GC Tm	**64.5**	**degrees C**
AT+GC Tm	**54.0**	**degrees C**
Absorbance	**5.7**	**nMol/A260**
Absorbance	**31.8**	**ug/A260**
Percent GC	**50.0**	**%**
Delta G	**-25.3**	**kCal/Mol**
Delta H	**-123.0**	**kCal/Mol**
Delta S	**-320.5**	**eu**
3' End Delta G	**-4.9**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 11 (LRMB primer 12S-L)" a 19-mer DNA Oligonucleotide (Sense)

5' CTA TTC GCC TCG CTC AGA C 3'

Oligonucleotide Analysis

Molecular weight	5779.8	
Tm thermodynamic	62.1	degrees C
Filter Tm	54.5	degrees C
% GC Tm	69.7	degrees C
AT+GC Tm	60.0	degrees C
Absorbance	6.0	nMol/A260
Absorbance	34.6	ug/A260
Percent GC	57.9	%
Delta G	-31.8	kCal/Mol
Delta H	-146.6	kCal/Mol
Delta S	-378.6	eu
3' End Delta G	-4.6	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 12 (LRMB primer 12S-H)" a 23-mer DNA Oligonucleotide (Antisense)

5' GCC TCC ATC ATC CCT CAC CTT AC 3'

Oligonucleotide Analysis

Molecular weight	6895.5	
Tm thermodynamic	70.8	degrees C
Filter Tm	63.2	degrees C
% GC Tm	75.3	degrees C
AT+GC Tm	72.0	degrees C
Absorbance	5.1	nMol/A260
Absorbance	34.9	ug/A260
Percent GC	56.5	%
Delta G	-38.9	kCal/Mol
Delta H	-174.6	kCal/Mol
Delta S	-448.9	eu
3' End Delta G	-5.1	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 13 (DTMB primer 16S-H)" a 20-mer DNA Oligonucleotide (Antisense)

5' CTC CGT CCG TCT CGC CTC TG 3'

Oligonucleotide Analysis

Molecular weight	6052.0
Tm thermodynamic	71.7 degrees C
Filter Tm	64.1 degrees C
% GC Tm	76.4 degrees C
AT+GC Tm	68.0 degrees C
Absorbance	6.1 nMol/A260
Absorbance	37.2 ug/A260
Percent GC	70.0 %
Delta G	-37.1 kCal/Mol
Delta H	-157.8 kCal/Mol
Delta S	-398.9 eu
3' End Delta G	-7.9 kCal/Mol

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 14 (DTMB primer 16S-L)" a 22-mer DNA Oligonucleotide(Sense)

5' AAA TCC GCC CTT ATG TGT GTT C 3'

Oligonucleotide Analysis

Molecular weight	6756.4
Tm thermodynamic	67.9 degrees C
Filter Tm	60.3 degrees C
% GC Tm	69.5 degrees C
AT+GC Tm	64.0 degrees C
Absorbance	4.9 nMol/A260
Absorbance	33.3 ug/A260
Percent GC	45.5 %
Delta G	-36.9 kCal/Mol
Delta H	-171.5 kCal/Mol
Delta S	-444.2 eu
3' End Delta G	-4.9 kCal/Mol

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs / palindromes	0 / 0
Number of hairpin loops	0
Number of dimers / 2-oligo dimers	0 / 0
Number of bulge loops / 2-oligo bulges	0 / 0
Number of internal loops / 2-oligo internals	0 / 0

Analysis of "table 15 (DTMB primer 12S-H)" a 22-mer DNA Oligonucleotide(Antisense)

5' CAT CGG CTT GCT CTA TTC CTT G 3'

Oligonucleotide Analysis

Molecular weight	**6723.4**	
Tm thermodynamic	**68.8**	**degrees C**
Filter Tm	**61.2**	**degrees C**
% GC Tm	**71.3**	**degrees C**
AT+GC Tm	**66.0**	**degrees C**
Absorbance	**5.3**	**nMol/A260**
Absorbance	**35.5**	**ug/A260**
Percent GC	**50.0**	**%**
Delta G	**-37.5**	**kCal/Mol**
Delta H	**-172.0**	**kCal/Mol**
Delta S	**-444.3**	**eu**
3' End Delta G	**-7.0**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 16 (DTMB primer 12S-L)" a 19-mer DNA Oligonucleotide (Sense)

5' TCT ATC GGC GGC GTA TCA C 3'

Oligonucleotide Analysis

Molecular weight	**5859.8**	
Tm thermodynamic	**65.8**	**degrees C**
Filter Tm	**58.2**	**degrees C**
% GC Tm	**69.7**	**degrees C**
AT+GC Tm	**60.0**	**degrees C**
Absorbance	**5.7**	**nMol/A260**
Absorbance	**33.4**	**ug/A260**
Percent GC	**57.9**	**%**
Delta G	**-33.9**	**kCal/Mol**
Delta H	**-152.5**	**kCal/Mol**
Delta S	**-391.2**	**eu**
3' End Delta G	**-3.5**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 17 (TCMB primer 16S-H)" a 21-mer DNA Oligonucleotide(Antisense)

5 ' GGC GAT TCT ACG GCA CGG GCG 3 '

Oligonucleotide Analysis

Molecular weight	6568.3	
Tm thermodynamic	80.4	**degrees C**
Filter Tm	72.8	**degrees C**
% GC Tm	78.6	**degrees C**
AT+GC Tm	72.0	**degrees C**
Absorbance	5.1	**nMol/A260**
Absorbance	33.3	**ug/A260**
Percent GC	71.4	**%**
Delta G	-44.7	**kCal/Mol**
Delta H	-186.4	**kCal/Mol**
Delta S	-468.6	**eu**
3' End Delta G	-12.8	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 18 (TCMB primer 16S-L)" a 22-mer DNA Oligonucleotide(Sense)

5' AAA CTG GTC CTC AAC TAT GTC A 3'

Oligonucleotide Analysis

Molecular weight	6758.5	
Tm thermodynamic	60.7	degrees C
Filter Tm	53.1	degrees C
% GC Tm	67.6	degrees C
AT+GC Tm	62.0	degrees C
Absorbance	4.7	nMol/A260
Absorbance	31.7	ug/A260
Percent GC	40.9	%
Delta G	-31.7	kCal/Mol
Delta H	-153.3	kCal/Mol
Delta S	-400.5	eu
3' End Delta G	-4.1	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 19 (TCMB primer 12S-H)" a 22-mer DNA Oligonucleotide(Antisense)

5' **CCG ATT CAG CCA CGA TTC CCT C** 3'

Oligonucleotide Analysis

Molecular weight	6671.4	
Tm thermodynamic	74.6	degrees C
Filter Tm	67.0	degrees C
% GC Tm	75.0	degrees C
AT+GC Tm	70.0	degrees C
Absorbance	5.1	nMol/A260
Absorbance	34.2	ug/A260
Percent GC	59.1	%
Delta G	-40.8	kCal/Mol
Delta H	-176.0	kCal/Mol
Delta S	-447.5	eu
3' End Delta G	-7.9	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 20 (TCMB primer 12S-L)" a 21-mer DNA Oligonucleotide (Sense)

5' **CCT AAA GCC CAG ATA ACT ACA** 3'

Oligonucleotide Analysis

Molecular weight	6432.3
Tm thermodynamic	59.2 degrees C
Filter Tm	51.6 degrees C
% GC Tm	66.9 degrees C
AT+GC Tm	60.0 degrees C
Absorbance	4.8 nMol/A260
Absorbance	30.6 ug/A260
Percent GC	42.9 %
Delta G	-31.7 kCal/Mol
Delta H	-159.4 kCal/Mol
Delta S	-421.0 eu
3' End Delta G	-3.9 kCal/Mol

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs / palindromes	0 / 0
Number of hairpin loops	0
Number of dimers / 2-oligo dimers	0 / 0
Number of bulge loops / 2-oligo bulges	0 / 0
Number of internal loops / 2-oligo internals	0 / 0

Analysis of "table 21 (PCMB primer 16S-H)" a 22-mer DNA Oligonucleotide(Antisense)

5' CGT GTT CTG ATG ATG ATG TGC T 3'

Oligonucleotide Analysis

Molecular weight	6867.5	
Tm thermodynamic	64.7	**degrees C**
Filter Tm	57.1	**degrees C**
% GC Tm	69.5	**degrees C**
AT+GC Tm	64.0	**degrees C**
Absorbance	4.9	**nMol/A260**
Absorbance	33.4	**ug/A260**
Percent GC	45.5	**%**
Delta G	-33.0	**kCal/Mol**
Delta H	-150.2	**kCal/Mol**
Delta S	-385.9	**eu**
3' End Delta G	-6.3	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs / palindromes	0 / 0
Number of hairpin loops	0
Number of dimers / 2-oligo dimers	0 / 0
Number of bulge loops / 2-oligo bulges	0 / 0
Number of internal loops / 2-oligo internals	0 / 0

Analysis of "table 22 (PCMB primer 16S-L)" a 19-mer DNA Oligonucleotide (Sense)

5' ATT CCT TCC TCT TAG TAT G 3'

Oligonucleotide Analysis

Molecular weight	5799.8	
Tm thermodynamic	49.5	**degrees C**
Filter Tm	41.9	**degrees C**
% GC Tm	61.1	**degrees C**
AT+GC Tm	52.0	**degrees C**
Absorbance	5.8	**nMol/A260**
Absorbance	33.6	**ug/A260**
Percent GC	36.8	**%**
Delta G	-26.1	**kCal/Mol**
Delta H	-138.8	**kCal/Mol**
Delta S	-371.5	**eu**
3' End Delta G	-3.1	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 23 (PCMB primer 12S-H)" a 22-mer DNA Oligonucleotide (Antisense)

5' GCT GAA CTT ACT ATG CCC TAC T 3'

Oligonucleotide Analysis

Molecular weight	6725.4	
Tm thermodynamic	60.3	degrees C
Filter Tm	52.7	degrees C
% GC Tm	69.5	degrees C
AT+GC Tm	64.0	degrees C
Absorbance	5.0	nMol/A260
Absorbance	33.6	ug/A260
Percent GC	45.5	%
Delta G	-32.7	kCal/Mol
Delta H	-164.7	kCal/Mol
Delta S	-435.2	eu
3' End Delta G	-6.6	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 24 (PCMB primer 12S-L)" a 20-mer DNA Oligonucleotide(Sense)

5' **CCG ATT GAC GCC GAA CTA TG** 3'

Oligonucleotide Analysis

Molecular weight	**6182.1**	
Tm thermodynamic	**68.1**	**degrees C**
Filter Tm	**60.5**	**degrees C**
% GC Tm	**70.3**	**degrees C**
AT+GC Tm	**62.0**	**degrees C**
Absorbance	**5.3**	**nMol/A260**
Absorbance	**32.5**	**ug/A260**
Percent GC	**55.0**	**%**
Delta G	**-35.6**	**kCal/Mol**
Delta H	**-159.4**	**kCal/Mol**
Delta S	**-408.5**	**eu**
3' End Delta G	**-4.1**	**kCal/Mol**

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 25 (SLMB primer 16S-H)" a 18-mer DNA Oligonucleotide(Antisense)

5' TAC GCA TAA CGG CTC TGG 3'

Oligonucleotide Analysis

Molecular weight	5579.7	
Tm thermodynamic	61.4	degrees C
Filter Tm	53.8	degrees C
% GC Tm	66.8	degrees C
AT+GC Tm	56.0	degrees C
Absorbance	5.9	nMol/A260
Absorbance	32.8	ug/A260
Percent GC	55.6	%
Delta G	-31.0	kCal/Mol
Delta H	-143.5	kCal/Mol
Delta S	-370.2	eu
3' End Delta G	-7.9	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 26 (SLMB primer 16S-L)" a 22-mer DNA Oligonucleotide (Sense)

5' CTA CTA CAC CTC AAC TAC ATC T 3'

Oligonucleotide Analysis

Molecular weight	6638.4
Tm thermodynamic	52.4 degrees C
Filter Tm	44.8 degrees C
% GC Tm	67.6 degrees C
AT+GC Tm	62.0 degrees C
Absorbance	4.9 nMol/A260
Absorbance	32.8 ug/A260
Percent GC	40.9 %
Delta G	-27.6 kCal/Mol
Delta H	-146.8 kCal/Mol
Delta S	-392.2 eu
3' End Delta G	-3.8 kCal/Mol

Analysis Parameters

Delta G Temperature	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 27 (SLMB primer 12S-H)" a 19-mer DNA Oligonucleotide(Antisense)

5' CCC ACT CAC TGC TAA CTC C 3'

Oligonucleotide Analysis

Molecular weight	5708.8	
Tm thermodynamic	58.4	degrees C
Filter Tm	50.8	degrees C
% GC Tm	69.7	degrees C
AT+GC Tm	60.0	degrees C
Absorbance	6.1	nMol/A260
Absorbance	35.0	ug/A260
Percent GC	57.9	%
Delta G	-29.4	kCal/Mol
Delta H	-138.5	kCal/Mol
Delta S	-359.0	eu
3' End Delta G	-5.4	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 28 (SLMB primer 12S-L)" a 21-mer DNA Oligonucleotide(Sense)

5' GGC TAA CTA CAA TCA TCT GCT 3'

Oligonucleotide Analysis

Molecular weight	6445.2	
Tm thermodynamic	58.5	degrees C
Filter Tm	50.9	degrees C
% GC Tm	66.9	degrees C
AT+GC Tm	60.0	degrees C
Absorbance	5.1	nMol/A260
Absorbance	32.6	ug/A260
Percent GC	42.9	%
Delta G	-30.8	kCal/Mol
Delta H	-153.4	kCal/Mol
Delta S	-403.9	eu
3' End Delta G	-6.3	kCal/Mol

Analysis Parameters

Delta G Temperature	25.0	degrees C
Probe concentration	0.6	pMol
Salt concentration	1000.0	mMol
Formamide concentration	0.0	%
3' End length	7	bases
Run length	4	bases
Palindrome length	8	bases
Hairpin loop stem length	3	bases

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		0
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0